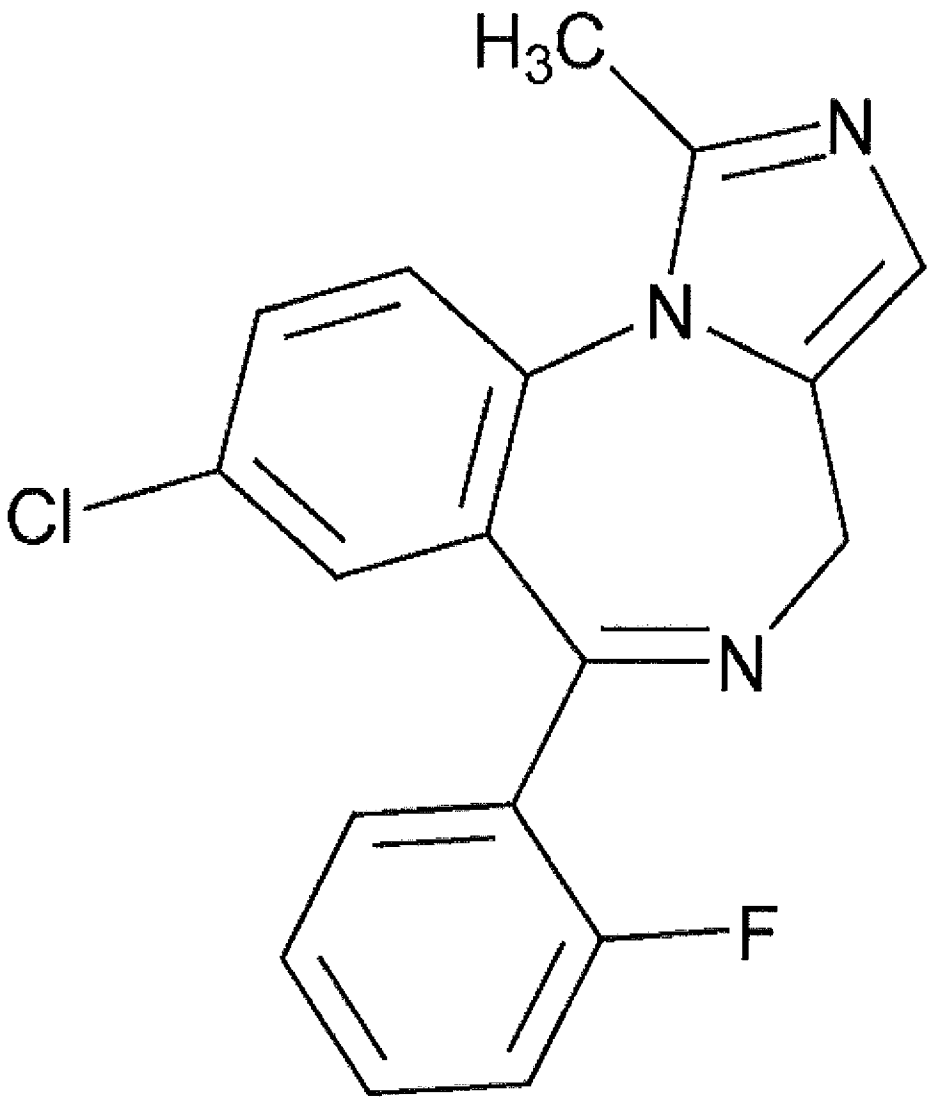
Midazolam



USP Image 3D Image

$C_{18}H_{13}ClFN_3$ 325.77

4-*H*-Imidazo[1,5-*a*][1,4]benzodiazepine, 8-chloro-6-(2-fluorophenyl)-1-methyl; 8-Chloro-6-(*o*-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine [59467-70-8].

DEFINITION

Midazolam contains NLT 98.5% and NMT 101.5% of $C_{18}H_{13}ClFN_3$, calculated on the dried basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** ⟨ 197K ⟩
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay.*

ASSAY

- **PROCEDURE**

Buffer: 7.7 g/L of ammonium acetate in water. Adjust with glacial acetic acid to a pH of 5.5 ± 0.1.

Mobile phase: Acetonitrile and *Buffer* (1:2)

Standard solution: 0.04 mg/mL of USP Midazolam RS in *Mobile phase*

Sample solution: 0.04 mg/mL of Midazolam in *Mobile phase*

Chromatographic system

(See *Chromatography* ⟨ 621 ⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; 5-µm packing L60

Flow rate: 1.5 mL/min

Injection size: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 10,000 theoretical plates

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_{18}H_{13}ClFN_3$ in the portion of Midazolam taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Midazolam RS in the *Standard solution* (mg/mL)

C_U = concentration of Midazolam in the *Sample solution* (mg/mL)

Acceptance criteria: 98.5%–101.5% on the dried basis

IMPURITIES

Inorganic Impurities

- **RESIDUE ON IGNITION** ⟨ 281 ⟩ : NMT 0.1%

Organic Impurities

- **PROCEDURE**

Buffer, Mobile phase, Standard solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Sensitivity check solution: Dilute the *Standard solution* with *Mobile phase* to obtain a 0.2-µg/mL solution.

Sample solution: 0.2 mg/mL of Midazolam in *Mobile phase*

System suitability

Samples: *Standard solution* and *Sensitivity check solution*

Suitability requirements

Column efficiency: NLT 10,000 theoretical plates, *Standard solution*

Tailing factor: NMT 2.0, *Standard solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Peak ratio: The ratio of the area of the midazolam peak of the *Standard solution* to the area of the midazolam peak of the *Sensitivity check solution* should be within 160–240.

Analysis

Sample: *Sample solution*

Calculate the percentage of each impurity in the portion of Midazolam taken:

$$\text{Result} = (r_U/F)/[\Sigma(r_U/F) + r_T] \times 100$$

r_U = peak response of each individual impurity from the *Sample solution*

r_T = peak response of Midazolam from the *Sample solution*

F = relative response factor (see *Impurity Table 1*)

Acceptance criteria: See *Impurity Table 1*.

Impurity Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Reduced midazolam[a]	0.20	1.0	0.1
Reduced reduced midazolam[b]	0.24	1.0	0.1
Amino compound[c]	0.25	0.5	0.1
Oxide midazolam[d]	0.46	1.3	0.1

Nitromethylene compound[e]	0.76	1.0	0.1
Dihydromidazolam[f]	0.83	0.5	0.1
Midazolam	1.0	—	—
Desfluoromidazolam[g]	1.14	1.0	0.2
6*H*-isomer[h]	2.48	0.7	0.1
Unknown impurity	—	1.0	0.1
Total impurities	—	—	0.5

[a] 8-Chloro-3a,4-dihydro-6-(2-fluorophenyl)-1-methyl-3*H*-imidazo[1,5-*a*][1,4]-benzodiazepine.

[b] 8-Chloro-6-(2-fluorophenyl)-3a,4,5,6-tetrahydro-1-methyl-3*H*-imidazo[1,5-*a*][1,4]-benzodiazepine.

[c] 2-Aminomethyl-7-chloro-2,3-dihydro-5-(2-fluorophenyl)-1*H*-1,4-benzodiazepine.

[d] 8-Chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*a*][1,4]-benzodiazepine-5-oxide.

[e] 7-Chloro-1,3-dihydro-2-nitromethylene-5-(2-fluorophenyl)-2*H*-1,4-benzodiazepine-4-oxide.

[f] 8-Chloro-6-(2-fluorophenyl)-5,6-dihydro-1-methyl-4*H*-imidazo[1,5-*a*][1,4]-benzodiazepine.

[g] 8-Chloro-6-phenyl-1-methyl-4*H*-imidazo-[1,5-*a*][1,4]-benzodiazepine.

[h] 8-Chloro-6-(2-fluorophenyl)-1-methyl-6*H*-imidazo[1,5-*a*][1,4]-benzodiazepine.

SPECIFIC TESTS

- **LOSS ON DRYING** ⟨ 731 ⟩: Dry a sample at 105° for 2 h: it loses NMT 0.5% of its weight.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in tight, light-resistant containers.
- **USP REFERENCE STANDARDS** ⟨ 11 ⟩
  USP Midazolam RS

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Mary S. Waddell Scientific Liaison 1-301-816-8124	(SM42010) Monographs - Small Molecules 4
Reference Standards	RS Technical Services 1-301-816-8129 rstech@usp.org	

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Chromatographic Column—

MIDAZOLAM

Chromatographic columns text is not derived from, and not part of, USP 34 or NF 29.

Midazolam Injection

DEFINITION

Midazolam Injection is a sterile solution of Midazolam Hydrochloride in Water for Injection or of Midazolam in Water for Injection prepared with the aid of Hydrochloric Acid. It contains the equivalent of NLT 90.0% and NMT 110.0% of the labeled amount of midazolam ($C_{18}H_{13}ClFN_3$). It may contain Sodium Chloride, Benzyl Alcohol, and/or a chelating agent.

IDENTIFICATION

- The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

[NOTE—Protect all prepared Standard and sample solutions from light.]

- **PROCEDURE**

Buffer: 6.7 g/L of dibasic sodium phosphate heptahydrate in water. Adjust with phosphoric acid to a pH of 5.0 ± 0.1.

Solution A: Prepare a filtered and degassed mixture of acetonitrile, methanol and *Buffer* (8:3:9).

Solution B: Acetonitrile and *Buffer* (3:1)

Mobile phase: See the gradient table below.

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	100	0
20	0	100
35	0	100
37	100	0
45	100	0

Standard solution: Dissolve USP Midazolam RS in about 2 mL of methanol, and dilute quantitatively, and stepwise if necessary, with *Solution A* to obtain a 0.2-mg/mL solution.

Sample solution: [NOTE—The midazolam present in the Injection converts from the open-ring form to the closed-ring form when diluted with *Solution A*. The midazolam potency is determined based on the peak area of the closed-ring form. It takes approximately 60 min at 40° or 2–3 h at room temperature to complete the conversion. The *Standard solution* is not subject to this conversion process.] Transfer a volume of Injection to a suitable volumetric flask, and dilute with *Solution A* to obtain a solution containing about 0.2 mg/mL of midazolam. Transfer the resulting solution into suitable

crimp top vials, seal tightly, and heat at about 40° for 60 min. Allow this solution to cool to room temperature before injection.

Chromatographic system

(See *Chromatography* ⟨ 621 ⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; packing L1

Flow rate: 1.0 mL/min

Injection size: 50 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Column efficiency: NLT 5500 theoretical plates

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of labeled amount of $C_{18}H_{13}ClFN_3$ in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of USP Midazolam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of Midazolam in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

IMPURITIES

Organic Impurities

[NOTE—Protect all prepared Standard and sample solutions from light.]

- **PROCEDURE**

Buffer, Solution A, Solution B, Mobile phase, Sample solution, and **Chromatographic system:** Proceed as directed in the *Assay*.

Standard stock solution: Use *Standard solution* in the *Assay*.

Standard solution: 0.5 µg/mL USP Midalozam RS in *Solution A* from *Standard stock solution*

Control solution: 0.1 µg/mL USP Midalozam RS in *Solution A* from *Standard solution*

System suitability

Samples: *Standard solution* and *Control solution*

Suitability requirements

Tailing factor: NMT 2.5 for midalozam peak, *Standard solution*

Column efficiency: NLT 5500 theoretical plates, *Standard solution*

Signal-to-noise ratio: NLT 10, *Control solution*

Relative standard deviation: NMT 8.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of the individual impurity from the *Sample solution*

r_S = peak response of midalozam from the *Standard solution*

C_S = concentration of USP Midalozam RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of Midalozam in the *Sample solution* (mg/mL)

F = relative response factor; 0.51 for the peak eluting at a relative retention between 0.79 and 0.97 with respect to midazolam; 1.0 for all other peaks

Acceptance criteria

Individual known impurity: NMT 0.5%

Individual unknown impurity: NMT 0.1%

Total impurities: NMT 1.0%

[NOTE—Disregard all solvent- and excipient-related peaks.]

SPECIFIC TESTS

- **BENZYL ALCOHOL CONTENT** (if present)

Buffer: 3.4 g/L of monobasic sodium phosphate in water. Adjust with phosphoric acid to a pH of 3.5.

Mobile phase: Acetonitrile and *Buffer* (7:13)

System suitability solution: 0.05 mg/mL of USP Midazolam RS and 0.5 mg/mL of USP Benzyl Alcohol RS in *Mobile phase*

Standard solution: 0.5 mg/mL of USP Benzyl Alcohol RS in *Mobile phase*

Sample solution: Transfer a measured volume of Injection to a suitable volumetric flask.

Dilute with *Mobile phase* to obtain a concentration of about 0.5 mg/mL of benzyl alcohol, based on the labeled content of benzyl alcohol in the Injection.

Chromatographic system

(See *Chromatography* ⟨ 621 ⟩, *System Suitability*.)

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; L1 packing

Flow rate: 1.0 mL/min

Injection size: 50 µL

System suitability

Sample: *System suitability solution*

Suitability requirements

Resolution: NLT 6.0 between benzyl alcohol and midazolam

Tailing factor: NMT 2.0 for benzyl alcohol

Relative standard deviation: NMT 2.0% for benzyl alcohol

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of benzyl alcohol in the volume of Injection taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of benzyl alcohol from the *Sample solution*

r_S = peak response of benzyl alcohol from the *Standard solution*

C_S = concentration of USP Benzyl Alcohol RS in the *Standard solution* (mg/mL)

C_U = nominal concentration of benzyl alcohol in the *Sample solution* (mg/mL)

Acceptance criteria: The content of benzyl alcohol meets the requirements for *Added Substances* under *Injections* ⟨ 1 ⟩.

- **PARTICULATE MATTER IN INJECTIONS** ⟨ 788 ⟩: Meets the requirements for small-volume injections
- **BACTERIAL ENDOTOXINS TEST** ⟨ 85 ⟩: It contains NMT 8.33 USP Endotoxin Units/mg of midazolam.
- **PH** ⟨ 791 ⟩: 2.5–3.7
- **STERILITY TESTS** ⟨ 71 ⟩: Meets the requirements
- **OTHER REQUIREMENTS:** It meets the requirements for *Injections* ⟨ 1 ⟩.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in single-dose containers, preferably of Type 1 glass. Store between 15° and 30°.
- **LABELING:** Label to indicate the vehicle used and the names and concentrations of any added preservatives. Indicate if the product is preservative free.
- **USP REFERENCE STANDARDS** ⟨ 11 ⟩
  USP Benzyl Alcohol RS
  USP Endotoxin RS
  USP Midazolam RS

Auxiliary Information— Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
Monograph	Mary S. Waddell Scientific Liaison 1-301-816-8124	(SM42010) Monographs - Small Molecules 4
⟨ 85 ⟩	Radhakrishna S Tirumalai, Ph.D. Principal Scientific Liaison 1-301-816-8339	(GCM2010) General Chapters - Microbiology
⟨ 71 ⟩	Radhakrishna S Tirumalai, Ph.D. Principal Scientific Liaison 1-301-816-8339	(GCM2010) General Chapters - Microbiology
Reference Standards	RS Technical Services 1-301-816-8129 rstech@usp.org	

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Chromatographic Column—

MIDAZOLAM INJECTION

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